Chapter III

GENERAL EXPERIMENTAL PROCEDURES
Zero order Spectrophotometric Procedures

a) Absorption spectrum of RPINH

5ml of buffer solution of required pH and appropriate volumes of DMF and the reagent solution are taken in a 10 ml volumetric flask. The total volume of the mixture is brought to 10 ml with distilled water. The absorbance of the solution is measured in suitable wavelength region against a blank consisting of 5 ml of buffer solution, appropriate volume of DMF and made up to the mark in 10 ml volumetric flask. A plot between absorbance and the wavelength is then made.

b) Absorption spectrum of the solution containing the complex species

In a 10 ml volumetric flask, 5 ml of buffer solution of desired pH and appropriate volumes of DMF, metal ion solution and reagent solution are taken. The contents of the flask are brought to the mark with distilled water. Its absorbance is measured in suitable wavelength region against the reagent blank prepared under identical conditions. A plot between absorbance and the wavelength is drawn from which the analytical wavelength is selected.

c) Effect of pH on the absorbance of the solution containing complex species

A known aliquot of metal ion and appropriate volumes of DMF and reagent solutions are taken in different 10ml volumetric flasks each containing 5 ml of buffer solution of different pH values. The contents of each flask are made up to the mark with distilled water and the absorbance of these solutions are measured against the corresponding reagent blank at the
analytical wavelength. A plot is then made between absorbance and pH, from which the working pH is chosen.

d) Effect of the reagent concentration

In a series of 10 ml volumetric flasks containing 5 ml buffer solution of desired pH, appropriate volume of DMF, different known aliquots of the reagent solution, a known aliquot of metal ion solution is taken and the contents are brought up to the mark with distilled water. The absorbance of the solution in each flask is measured against the corresponding reagent blank at the analytical wavelength, from which the required molar excess of the reagent necessary for maximum colour formation is ascertained.

e) Effect of DMF volume on the absorbance

The reagent and many of its metal complexes are insoluble in aqueous medium. But it is observed that the reagent and its complexes are soluble in aqueous DMF. In order to arrive at the optimum percentage of DMF required to retain them in solution, the following procedure is adopted.

To different 10 ml volumetric flasks each containing 5 ml of buffer solution, appropriate volume of metal ion solution, required aliquots of reagent solution and different volumes of DMF are added. The contents of the flasks are made up to the mark with distilled water and the absorbance of these solutions is measured at the selected analytical wavelength against the reagent blank.
f) Effect of time on the colour development and on the stability of the colour

5 ml of buffer solution, required volume of DMF, an aliquot of metal ion and the reagent solutions are taken in a 10 ml volumetric-flask and made up to the mark with distilled water. The absorbance of the solution is measured at different time intervals at the selected wavelength against the reagent blank from which the time interval required to be allowed after mixing various components of the reaction mixture and before measuring the absorbance is known.

g) Adherence of the systems to Beer's law\(^1\)

To ascertain the sensitivity of the colour reactions and to explore the possibility of determining micro amounts of metal ions, the following procedure is adopted.

Varying known aliquots of metal ion solutions are added to a set of 10ml volumetric flasks, each containing 5 ml of buffer solution of desired pH, known volume of DMF and the necessary excess of the reagent solution. The contents of the flasks are brought up to the mark with distilled water and the absorbance of the solutions are measured at the analytical wavelength against the reagent blank. A plot of absorbance and amount of metal ion (µg/ml) is constructed. The slope and the intercept of the plot are computed. The molar absorptivity is calculated from the slope.
h) Effect of foreign ions

In order to assess the applicability of the proposed methods for analysis of real samples containing the metal ions, the effect of the presence of various foreign ions which are generally found associated with the test metal ions in various real samples on the absorbance of the reaction mixture is studied by adopting the following procedure.

To different 10 ml volumetric flasks, each containing an interfering ion of known amount and desired volume of DMF, 5 ml buffer solution of desired pH, known aliquots of metal ion and the reagent solutions are added. The contents are brought up to the mark with distilled water. The absorbance is measured at the analytical wavelength against reagent blank from which the tolerance limit of the foreign ion is determined. The amount of foreign ion which brings about a change in absorbance by ± 2% is taken as its tolerance limit.

i) Composition and stability constant of the complex species

The composition of complex species is determined by Job’s method and mole ratio methods.

Job’s method

To a series of 10 ml volumetric flasks, each containing 5 ml of buffer solution of desired pH and known volume of DMF, equimolar solutions of metal ion and the reagent are added in different volume proportions, such that the total volume of the mixture is held constant. The absorbance of each solution is measured at the wavelength of maximum absorbance or at an appropriate wavelength against buffer blank. A plot between mole fraction of
the metal ion \( \frac{V_M}{V_M + V_I} \) or \( \frac{C_M}{C_M + C_I} \) and the absorbance is made from which the composition of the complex is computed.

j) **Determination of stability constant of the metal complexes**

The spectrophotometric data obtained in the study of complexes using Job's method is used to calculate the stability constants of the complex species under investigation.

For the complex formation reaction

\[
mM + nL \rightarrow M_mL_n \tag{3.1}
\]

The stability constant \( \beta \) in terms of experimentally obtained absorbance value is given by the general equation.

\[
\beta = \frac{\frac{A}{A_m}}{m^m n^n \left[ \frac{1-A}{A_m} \right]^{m+n} [C]^{m+n-1}} \tag{3.2}
\]

Where

- \( A_m \) = absorbance corresponding to the point of intersection of the extrapolated lines
- \( A \) = observed absorbance at concentration \( C \)
- \( C \) = concentration corresponding to the point of intersection
- \( \beta \) = stability constant and

\[
\alpha = \frac{A_m - A}{A_m} \quad \text{Where} \quad \alpha = \text{degree of dissociation}
\]

k) **Mole ratio method**

5 ml of buffer solution and known volumes of DMF are taken in a series of 10 volumetric flasks and known volume of metal ion solution and varying
volumes of the reagent solution are added. The contents of each flask are brought up to the mark with distilled water. The absorbance of each solution is measured at the selected wavelength against the corresponding reagent blank or a blank containing 5 ml of buffer solution and required volume of DMF. From the plot between the absorbance and the volume of the reagent, the composition of the complex is ascertained.

**Derivative spectrophotometric procedures**

**I) Derivative spectrum of the solution containing the complex species**

In a 10 ml volumetric flask, 5 ml of buffer solution of desired pH and appropriate volumes of DMF, metal ion solution and the reagent solution are taken. The contents of the flask are brought to the mark with distilled water. Its absorption spectrum in a suitable wavelength region is recorded against the reagent blank prepared under identical conditions. Then the first and second derivative spectra are recorded. From these analytical wavelengths are fixed.

**m) Effect of pH on the derivative amplitude**

A known aliquot of metal ion and appropriate volumes of DMF and the reagent solution are taken in different 10 ml volumetric flasks each containing 5 ml of buffer solution of different pH values. The contents of each flask are made up to the mark with the distilled water the absorption spectrum of these solutions are recorded against the corresponding reagent blank and the first and second derivative spectra are recorded. A plot is then made between the derivative amplitude and pH from which the working pH is chosen.
n) Effect of the reagent concentration on the derivative amplitude

In a series of 10 ml volumetric flasks containing 5 ml buffer solution of desired pH appropriate volume of DMF, different known aliquots of the reagent solution; a known aliquot of metal ion solution is taken and the contents are brought up to the mark with distilled water. The absorption spectrum and consequently the first and second derivative plots of the solution in each flask are recorded against the corresponding reagent blank. The derivative amplitudes at the selected wavelengths are measured from which the required molar excess of the reagent necessary for maximum colour formation is ascertained.

o) Effect of DMF volume on the derivative amplitude

The reagent and many of its metal complexes are insoluble in aqueous medium. But it is observed that the reagent and its complexes are soluble in aqueous DMF. In order to arrive at the optimum percentage of DMF required to retain them in solution, the following procedure is adopted.

To different 10 ml volumetric flasks each containing 5 ml of buffer solution appropriate volume of metal ion solution required aliquots of reagent solution and different volume of DMF are added. The contents of the flask are made up to the mark with distilled water and the derivative amplitudes of these solutions are measured at the selected analytical wavelength against the reagent blank.
p) Effect of time on the derivative amplitude

5 ml of buffer solution, required volume of DMF, an aliquot of metal ion and the reagent solutions are taken in a 10 ml volumetric flask and made up to the mark with distilled water. The derivative amplitudes of the solution are measured at different time intervals at the selected wavelength against the reagent blank from which the time interval required to be allowed after mixing various components of the reaction mixture and before measuring the derivative amplitude is known.

q) Adherence of the systems to Beer's law (calibration plot)

To ascertain the sensitivity of the colour reaction and to the possibility of determining micro amounts of metal ions the following procedure is adopted.

Varying known aliquots of metal ion solutions are added to a set of 10ml volumetric flasks each containing 5 ml of buffer solution of desired pH. Known volume of DMF and the necessary excess of reagent solution. The contents of the flasks are brought up to the mark with distilled water and the derivative amplitudes of the solutions are measured at the analytical wavelengths against the reagent blank. Plots of derivative amplitudes and amount a metal ion (µg/ml) are constructed. The slope and the intercept of these plots are computed.

r) Effect of foreign ions

In order to assess the applicability of the proposed methods for analysis of real samples containing the metal ions, the effect of the presence
of various foreign ions which are generally found associated with the test metal ions in various real samples on the absorbance of the reaction mixture is studied by adopting the following procedure.

To different 10 ml volumetric flasks, each containing an Interfering ion of known amount and desired volume of DMF, 5 ml buffer solution of desired pH known aliquots of metal and the reagent solutions are added. The contents are brought of to the mark with distilled water. The derivative amplitudes are measured at the analytical wavelength against reagent blank from which the tolerance limit of the foreign ion is determined. The amount of foreign ion, which brings about a change in the derivative amplitudes by ± 2% is taken as its tolerance limit.

s) Preparation of alloy, steel fly and industrial samples

A 0.1 - 0.5 g of the sample is dissolved in a mixture of 2 ml HCl and 10 ml HNO₃. The resulting solution is evaporated to a small volume. To this E ml of 1:1 H₂O : H₂SO₄ mixture is added and evaporated to dryness. The residue is dissolved in 15 ml of distilled water and filtered through Whatman filter paper No. 41. The filtrate is collected in a 100 ml volumetric flask and made up to the mark with distilled water. The solution is further diluted as required.

t) Preparation of plant and other biological material samples

Freshly collected samples are cleaned and dried for one hour in open air protecting from mineral contamination. The dried sample is powdered in a mortar for the purpose of analysis. The powdered material is brought into solution either by wet ashing or dry method according to the procedure given in the literature⁴.
u) **Wet ashing**

Weighed quantity of finely powdered sample is transferred into a 100ml beaker. To it, 20 ml mixture 1:2:5 of H$_2$SO$_4$, H$_3$PO$_4$ and HNO$_3$ are added. The contents are heated until sulfurous acid fumes are evolved and the volume is reduced to about 5 ml. A little distilled water is added and filtered through an acid washed Whatman filter paper No. 41 into a volumetric flask and made up to the mark.

v) **Dry ashing**

A known quantity of the powdered sample is taken in a silica crucible and heated to oxidize organic matter. It is ashed at 550°C by keeping for 3-4 hours in a muffle furnace. The ash is dissolved in 10 ml of 1:1 HCl by heating, filtered through an acid washed filter paper into a volumetric flask and the residue is washed with water. The washings are also collected into volumetric flask and finally made up to the mark with distilled water. The solution is then diluted according to the requirement.
REFERENCES